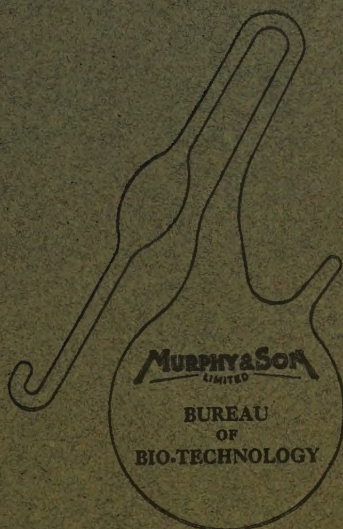

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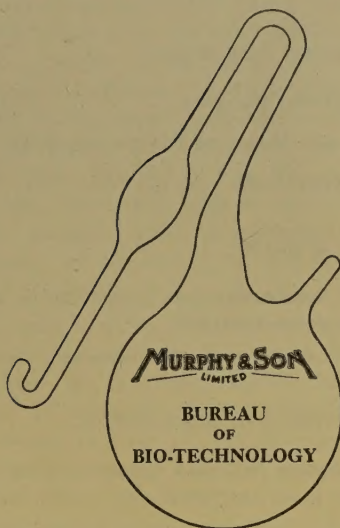
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Acetifying Bacteria

THE GENUS *ACETOBACTER* AND ITS RELATIONSHIPS

ALL the known members of this genus possess special interest for those engaged in the fermentation industries. Each species is capable of producing acetic acid from alcohols and various sugars in solution, and whilst some of them are of the greatest possible use in vinegar manufacture, their presence in the products of the brewer is a source of grave detriment. The danger to the latter lies in the fact that so small a quantity as 0.2 per cent. of acetic acid is sufficient to render beer unsaleable, and bacterial action once started may continue until at least ten times that amount of acid has been produced.

The various acetifying organisms have been known in the past under the generic name, *Bacterium*, and brewers are familiar with the names *Bacterium aceti*, *B. xylinum*, *B. pasteurianum*, etc., either from their own laboratory studies, or in the literature of their industry. In the most recent classification of the bacteria, however, *Bacterium*, as a genus, has disappeared, and has been replaced by a series of names of recent introduction, or by older names that have fallen into disuse.

An article printed in the first issue of this Bulletin, July 1920, dealt with "System in the Nomenclature of the Bacteria," and partook of the nature of a plea for greater uniformity in the classification of organisms belonging to the great class, Schizomycetes, in which reference was made to important work then in progress by a Committee of the American Society of Bacteriologists. Since that time a vast amount of labour has been expended in researches aimed at producing a practical system of classification comparable with those of other groups which have long been available to botanists and zoologists, and the recent publication

of a "Manual of Determinative Bacteriology" marks a great advance in the systematics of the subject. In spite of systems previously devised by Cohn, Winter, Zopf, Flügge, Sternberg, Migula, and more recently, Orla-Jensen, the greatest difficulty the industrial bacteriologist had to face was that of nomenclature. It was well nigh impossible for a serious worker to attach a name to any bacterium other than the well-studied animal pathogens. A few saprogens, certainly, such as *B. subtilis*, *B. mycoides* and *B. prodigiosus* are readily recognisable, but after weeks and months of study devoted to organisms commonly met with in beer, cider, soil, air-infected wort and must, etc., it required irrepressible optimism to maintain faith in any names with which it might be decided to label them. Thanks to the work of contemporary American bacteriologists, among whom must be mentioned Berger, Buchanan, Conn, Enlows, the Winslows, and Erwin F. Smith, that period of frightfulness has abated and the bacteriologist is now placed in possession of a key to some of his most difficult problems.

It is not to be implied that one has now only to look through the microscope and by means of the Manual read off the name of any bacterial organism that may be in view. Experience in bacteriological technique is more than ever necessary, and only by carefully conducted examination of an organism is it possible to arrive at the same conclusions as the Committee which has taken upon itself the task of nomenclature.

The following notes are designed to assist brewing students and those who have not followed recent developments along this line, as well as vinegar brewers whose interest is limited to certain acetic acid producing organisms, which, important as they are to those concerned, are inconspicuous in a Manual dealing with all the better described species of bacteria. The descriptive matter is abstracted directly from the Manual but it is presented in a more graphic form intended to focus on the genus under consideration.

The broad differences between Orders, Families, Tribes and Genera are included to give perspective to the group, and the names printed in bolder type indicate the steps which lead to the group under consideration.

Table I.

CLASS SCHIZOMYCETES (Nägeli) MIGULA.

Typically unicellular plants, cells usually small and relatively primitive in organization. The cells are of many shapes, spherical, cylindrical, spiral or filamentous; cells often united into groups, families or filaments; occasionally in the latter showing some differentiation among the cells, simulating the organization seen in some of the blue-green, filamentous algæ. Multiplication typically by cell fission. Endospores are formed by some species of the Eubacteriales, conidia by some of the filamentous forms. Chlorophyll is formed by none of the bacteria (with the possible exception of a single genus). Many forms produce pigments of other types. The cells may be motile by means of flagella: some of the forms intergrading with the protozoa are flexuous, a few filamentous forms (as *Beggiatoa*) show oscillatory movement similar to that of certain blue-green algæ (as *Oscillatoria*).

Key to the orders of the class Schizomycetes (Buchanan).

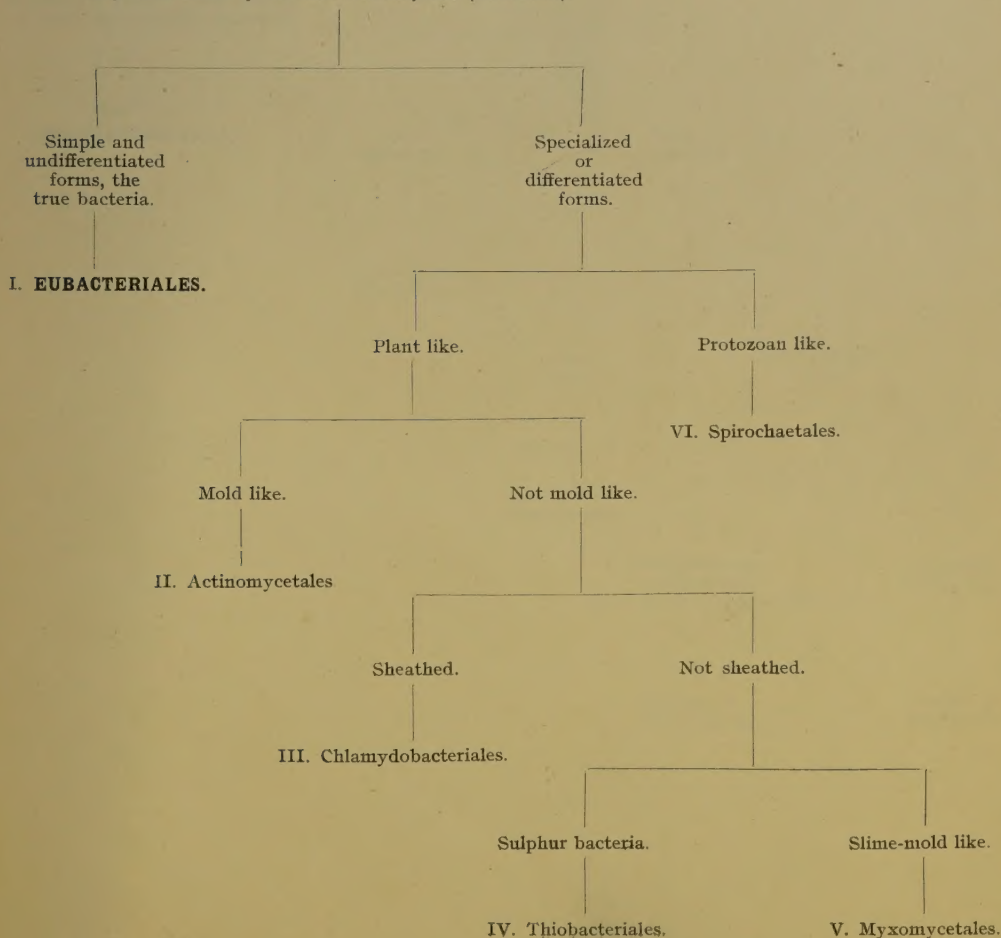
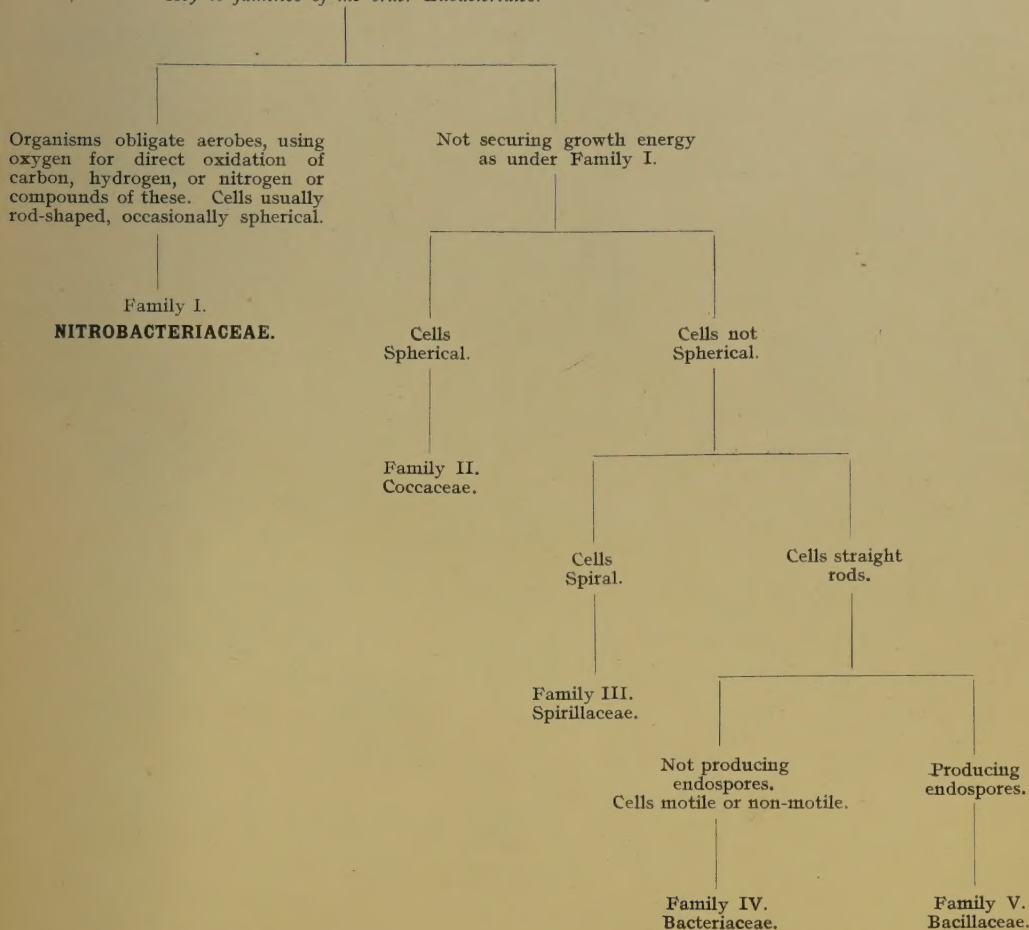


Table II.

ORDER I. **EUBACTERIALES** BUCHANAN, 1917.

Key to families of the order Eubacteriales.



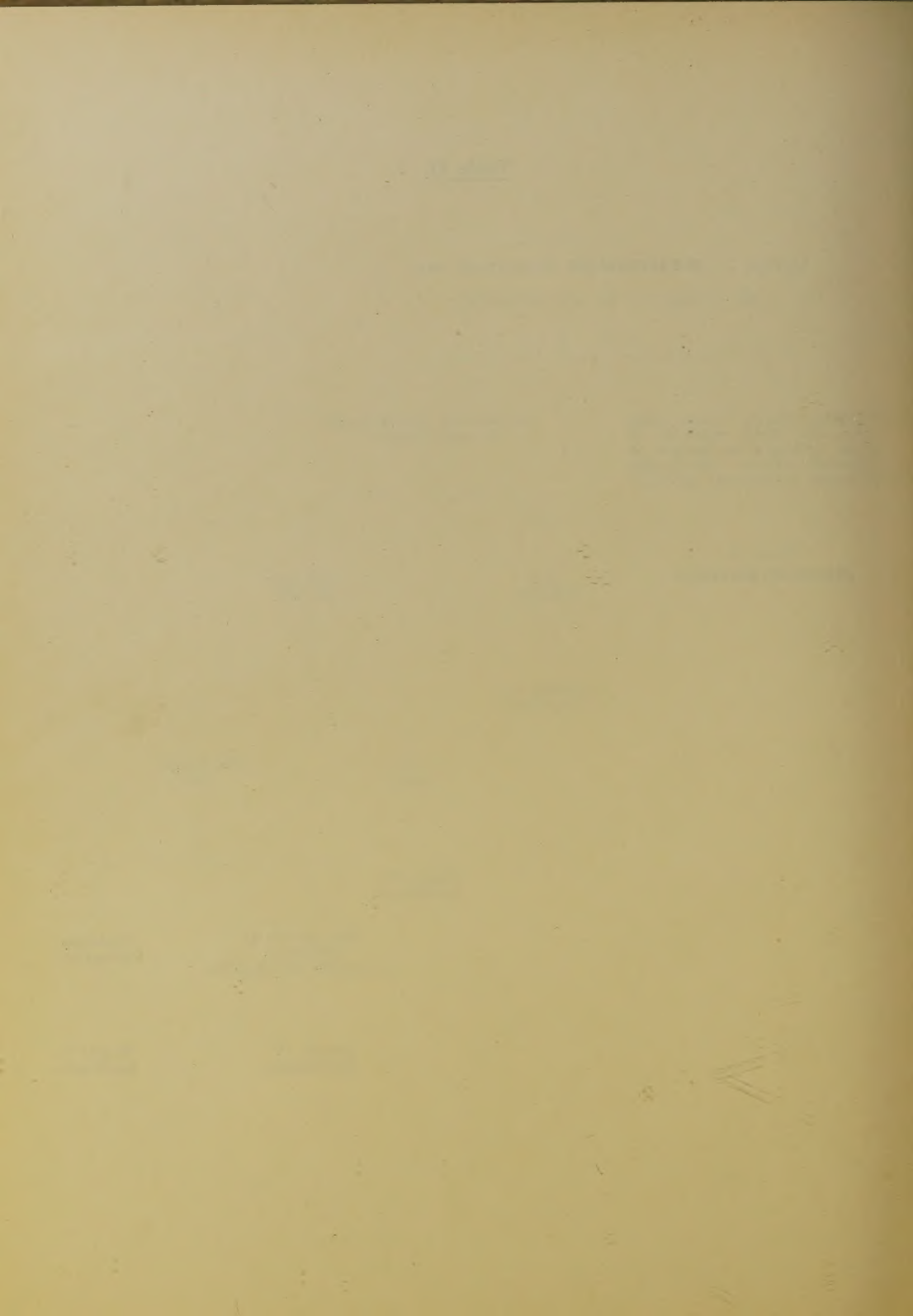


Table III.

FAMILY I. NITROBACTERIACEAE BUCHANAN, 1917.

Organisms usually rod-shaped, sometimes nearly spherical. Motile or non-motile. Branched involution forms are produced. Endospores never formed. Obligate aerobes, capable of securing energy by direct oxidation of carbon, hydrogen or nitrogen or of simple compounds of these. Non-parasitic (except in genus *Rhizobium*), usually water or earth forms.

Key to the tribes and genera of the family Nitrobacteriaceae.

Organisms oxidize simple compounds of carbon and nitrogen.

Tribe I.

Nitrobacterace.

Cells capable of securing growth energy by the oxidation of hydrogen to form water.

Genus I.
Hydrogenomonas.

Cells oxidize methane to form CO₂ and water.

Genus II.
Methanomonas.

Cells oxidize CO to form CO₂.

Genus III.
Carboxydomonas.

Cells oxidize ammonia to form nitrates.

Genus IV.
Nitrosomonas.

Cells oxidize nitrites to nitrates.

Genus V.
Nitrobacter.

Cells oxidize alcohol to form acetic acid.

Genus VI.
ACETOBACTER.

Cells oxidize compounds of sulphur.

Genus VII.
Thiobacillus.

Organisms capable of fixing free nitrogen of the air.

Tribe II.

Azotobacterace Committee S.A.B., 1917.

Cells capable of fixing free atmospheric nitrogen when growing in solutions of carbohydrates.

Genus VIII.
Azotobacter.

Cells capable of fixing free nitrogen when growing symbiotically on the roots of Leguminosae.

Genus IX.
Rhizobium.

Table IV.

GENUS VI. **ACETOBACTER** FUHRMANN, 1905.

Cells rod-shaped, frequently in chains, non-motile. Usually grow on the surface of alcoholic solutions as obligate aerobes, securing growth energy by the oxidation of alcohol to acetic acid. Also capable of utilizing certain other carbonaceous compounds, as sugar and acetic acid. Elongated, filamentous, club-shaped, swollen and even branched cells may occur as involution forms.

The type species is *Acetobacter aceti* (Thomsen) Committee S.A.B., 1920.

Key to the species of genus Acetobacter.

Form acetic acid
in dextrose,
ethyl and propyl
alcohol,
and in glycol.

1. *Acetobacter aceti*.
2. *Acetobacter acetigenus*.
3. *Acetobacter kützingianus*.
4. *Acetobacter pasteurianus*.
5. *Acetobacter lindneri*.

Form acetic acid
in dextrose,
ethyl and propyl
alcohol,
glycol, and in
galactose.

6. *Acetobacter acetosus*.

Form acetic acid
in dextrose,
ethyl and propyl
alcohol,
glycol, and in
sucrose.

7. *Acetobacter xylinus*.

Form acetic acid
in dextrose,
ethyl and propyl
alcohol, glycol,
arabinose,
levulose, maltose,
lactose,
raffinose, dextrin,
glycerol and
mannitol.

8. *Acetobacter oxydans*.
9. *Acetobacter industrius*.

Form acetic acid
in ethyl and
propyl alcohol
and in glycol.

10. *Acetobacter ascendens*.

Table V.

DISTINGUISHING CHARACTERISTICS OF THE SPECIES OF ACETOBACTER.

NAME ADOPTED BY COMMITTEE S.A.B.	<i>A. aceti</i>	<i>A. acetigenus</i>	<i>A. kützingianus</i>	<i>A. pasteurianus</i>	<i>A. lindneri</i>	<i>A. acetosus</i>	<i>A. xylinus</i>	<i>A. oxydans</i>	<i>A. industrius</i>	<i>A. ascendens</i>
SYNONYMS	<i>Mycoderma aceti</i>	<i>Bacterium acetigenum</i> (Henneberg)	<i>B. kützingianum</i> (Hansen)	<i>B. pasteurianum</i> (Hansen)	<i>B. lindneri</i> (Zeidler)	<i>B. acetosum</i> (Henneberg)	<i>B. xylinum</i> (Brown)	<i>B. oxydans</i> (Henneberg)	<i>B. industrium</i> (Henneberg)	<i>B. ascendens</i> (Henneberg)
MORPHOLOGICAL FEATURES	Rods : 0.4-0.8 μ - 1.0-2.0 μ occurring singly and in long chains, frequently showing large, club-shaped forms	Rods : occurring singly and in pairs	Short thick rods occurring singly	Rods : 0.4-0.8 μ - 1.0 μ occurring singly and in chains, at times showing thick club-shaped forms	Rods : occurring singly and in chains, showing large sausage-shaped involution forms	Rods : 0.4-0.8 μ - 1.0 μ occurring singly and in chains	Rods : occurring singly and in chains The cells have a slimy envelope which gives a	Rods : 0.8-1.2 μ - 2.4-2.7 μ occurring singly and in chains. The chains show bud-like swellings	Rods : 0.3-0.8 μ - 2.4-20 μ	Rods : occurring singly, rarely in chains
STAINING REACTIONS	Stain yellow with iodine	Cellulose reaction with H ₂ SO ₄					Cellulose reaction			Does not give the cellulose reaction
CULTURAL FEATURES	Forms large shining colonies on beer gelatine containing 10 per cent. sucrose Slimy pellicle on fluid media	Dextrose gelatine colonies : Raised, greyish, slimy Fluid cultures show a tough slimy pellicle	In beer gelatine the colonies are slimy A moist, smooth film develops on fluid media, tending to rise on the sides of the flask	Wort gelatine colonies : Small, entire, with vermiciform surface. Forms a thick, folded pellicle on fluid media	Wort gelatine colonies : Small, circular, slightly granular, yellowish-brown, entire, Dirty yellowish-brown pellicle on fluid media		Forms a film on beer which becomes cartilaginous and falls to the bottom	Gelatine colonies : Circular, becoming irregular in shape with peculiar ramifications		Glucose gelatine colonies : Dry, white, with white area surrounding the colony. Fluid cultures have a tough pellicle rising on the walls of the flask
OXYGEN RELATIONSHIP	Aerobic	Aerobic		Aerobic	Aerobic, facultative		Aerobic	Aerobic	Aerobic	Aerobic
OPTIMUM TEMPERATURE	30°C	33°C.	30°C.	25°C.	25°C.	30°-36°C.	30°C.	18°-21°C.	25°C.	25 C.
HABITAT	Vinegar	Vinegar	Beer	Beer and beer-wort	Beer-wort	Beer	Vinegar	Beer	Beer-wort	Isolated from vinegar and from red wine
REFERENCES	Annalen der Chemie und Pharmazie, 1852, 89 J. 95 K. 336 L. 549	Centralblatt f. Bakt., II. Abt., IV., 1898, 14 J. 101 K. 340 L. 553	Compt. Rend. Carlsberg Lab., 1894, 265 J. 96 K. 338 L. 549	Compt. Rend. Carlsberg Lab., 1879 J. 96 K. 337 L. 549	Centr. f. Bakt., II. Abt., 1896, 729 L. 523	Centr. f. Bakt., II. Abt., IV., 1898, 14 J. 101 K. 319 L. 553	Journ. Chem. Soc. London, XLIX., 1886, 432 J. 100 K. 340 L. 553	Centr. f. Bakt., II. Abt., III., 1897, 223 J. 100 K. 340 L. 553	Zeitschr. f. Deutsche Essig-ind., 1898 Centr. f. Bakt., II. Abt., IV., 1898, 933 J. 101 K. 340 L. 553	Centr. f. Bakt., II. Abt., IV., 1898, 923 J. 102 L. 553

Table V. gives all the information available so far as it is useful in aiding the separation of the species, and the specific differences there shown are bound to be taken into consideration in any subsequent work on the important physiological aspects of the acetifying organisms before that work can possess permanent value. A great many gaps in our knowledge of the group still remain to be filled, and those who should best be able to fill them are those engaged in the industries from which these organisms are inseparable.

Having regard to the new requirements of the Institute of Brewing for its Diploma qualification, more frequent reference to the acetifying bacteria is likely to be made than has been thought to be necessary in the past. In all pre-existing literature species will be located under the name given as a synonym in the Table.

The first item under "References" applies to the original description of the species by its discoverer. For the convenience of those to whom the original source is only with difficulty accessible, references to the works more commonly in use in brewers' and other works' laboratories have been added. These are denoted by initial letters followed by a page number, and they refer to the following texts, each of which gives information relative to the particular species:

- J. — Alfred Jörgensen: *Micro-Organisms and Fermentation*. Eng. Trans., London, 1911.
- K. — Alb. Klöcker: *Fermentation Organisms: a Laboratory Hand-book*. Eng. Trans., London, 1903.
- L. — Paul Lindner: *Mikroskopische Betriebskontrolle in den Gärungsgewerben*. Berlin, 1909.

F. A. M.

The Red Mould of Barley and Malt

WITH SOME NOTES ON RELATED FUNGI

ALL maltsters are familiar with the pink, or salmon-coloured mould-growths which make their appearance on germinating barley on the growing floor, usually near the time that the acrospire is showing about half-way up the back. They are first seen on the endosperm of broken corns—corns with snapped ends, or those otherwise damaged in threshing; less frequently, on the husk of whole corns, as white woolly tufts, later becoming pink, or, sometimes yellow. If, at this stage of development, such tufts are examined under the microscope, they are seen to consist entirely of branching, septate, mycelial threads, or aerial hyphæ, with no indication of fructification. At a later stage in the malting process the downy tufts take on a more gelatinous appearance, and, finally, at the withering stage, the fluffiness entirely disappears and small, granular, closely compacted, deeper-coloured tuberculate granules are formed, which remain strongly adherent to the testa of the grain. After drying on the kiln the same fungus arrests the eye of the brewer as a minute red excrescence of a horny texture. Examined microscopically in that condition, a tubercle, detached from its matrix, will be found to be composed of enormous numbers of large, more or less crescent- or sickle-shaped, septate conidia, usually showing comparatively few filaments, which will be readily recognised as belonging to some species of *Fusarium*.

By relatively simple observations the life-history of various *Hyphomycetes* of the genus *Fusarium* may be traced. The size and readily germinative character of the conidia render them particularly suitable for study by moist chamber methods under

the microscope, and many years ago conidia collected from the grain on malting floors were used in these laboratories for that purpose in teaching biology to brewing students. Those conidia which germinate on the sprouting grains are derived from sporodochia previously produced on ears of barley parasitized by the fungus in the field, and which have been scattered among the grain at threshing. On germination, the septate conidium produces one to several mycelial threads from any one, or each, of the separate cells formed by the septa. The result of a few days growth (or even a few hours, according to the temperature at which observations are made) is an interwoven, much-branched septate mycelium. A feature of the mycelium is the production of lateral branches, bearing *chlamydospores*, which are usually intercalary, or rarely terminal. In different species the chlamydospores may be developed singly, in chains, or in groups. Two forms of conidium are recognised; *microconidia*, which are small, oval, non-septate, or occasionally one to two-septate cells, bearing little resemblance to the *macroconidia* which are the larger, pluriseptate, crescent- or sickle-shaped (falcate) cells characteristic of the genus. The latter are described as dorsi-ventral, alternate and pedicellate; they are smooth, not constricted at the septa, and are produced acrogenously.

In the natural state, as well as in pure culture, vast numbers of conidia are developed in either slimy layers, or in dry, wart-like patches. In the latter and more usual form the fructification is a *sporodochium*, whilst the slimy formation is known as *pionnotes*. These are the terms used by Appel and Wollenweber (1) who first monographed the genus. The production of pionnotes in the strict sense of the term is denied by Sherbakoff (2) who uses the term *pseudopionnotes*. Sherbakoff says, in connection with his work on the *Fusaria* of potatoes, that "the conidia are formed in more wart-like heaps when the air is moist, whereas when the air is comparatively dry the conidia are pushed up into more or less long, tendril-like columns." These observations are supported by what is seen on a malting floor where the grain contains, according to the stage of its growth, from 15 to 50 per cent. of water and the conidial growths are always typical sporodochia.

The colour of the aerial hypæ and of the macroconidia seen *en masse*, is always bright, varying in colour from yellow, orange, or pink to dark red and purple.

Attention was first directed to the occurrence of red mould in maltings by the well-known Burton fermentation chemist, C. G. Matthews, in a communication to the Royal Microscopical Society, published in 1883. (3) Matthews described the fungus under the name, *Fusarium graminearum*. Later, in 1884, Worthington G. Smith renamed what he supposed was the same fungus, *Fusisporium hordei*, and published an account of the organism under that name in his "Diseases of Field and Garden Crops," in a chapter devoted to "New Diseases of Wheat, Barley, and Rye-Grass by *Fusisporium culmorum*, *hordei*, and *Lolii* W. Sm."

In a paper by Frank E. Lott, entitled "Experiments on the apparent effect of Mould on Malt, as shown in the composition of the Extract and Beer brewed from Mouldy Malt," published in 1899, (4) reference is made to the fact that Lindner "considers that *Fusarium hordei* is identical with a mould producing similar peculiar crescent-shaped spores, and growing in small red patches on malt, described by Kitasato under the name of *Fusisporium moschatum*, but as this latter is especially noticeable as giving an intense odour of musk when cultivated on wort gelatine, and *Fusarium hordei* gives no such odour, it is undoubtedly a distinct species, and one which, so far as I am aware, has not been noticed on English malts."

In G. Massee's "Text book of Plant Diseases," 1903, the "red mould of barley" is referred to under another name, *Fusarium heterosporum* Nees. Here Massee happened upon a name which is synonymous with the correct one, but that this fact was unknown to him is evident from his procedure of bracketing it with *F. Lolii*, which is another species.

In "Mikroskopische Betriebskontrolle in den Gärungsgewerben," 1909, Lindner adheres to the name *Fusisporium moschatum* Kitasato, and gives sketches of the germinating conidia showing the production of microconidia and macroconidia, and further suggests that the fungus may also be identical with *Selenosporium aquaeductum* Lagh.

The *Fusaria*, in 1910, were the subject of an important monograph by Appel and Wollenweber, (1) who completely reconstructed the genus and laid the foundations on which all subsequent systematic work on this economically important group of fungi is based.

C. D. Sherbakoff, in 1915, published a monograph, "*Fusaria* on Potatoes," (2) in which he described forty species and varieties obtained from potatoes. It contains cultural descriptions of *F. arthrosporioides* Sherb., *F. culmorum* (W. G. Sm.) Sacc., *F. arcusporium* Sherb., *F. redolens* Wr.—all fungi known to parasitize cereals as well as potatoes.

D. Atanasoff, in 1920, published a paper entitled "*Fusarium*-blight (scab) of Wheat and Cereals," (5) a work followed in 1923 by a monograph on "*Fusarium* Blight of Cereal Crops," (6) to which further reference will be made.

Although the conidia of *Fusarium* are among the most easily recognisable objects which a fermentation biologist may be called upon to identify, the separation of the species to which they belong is a task upon which even the experienced mycologist may not embark lightly. The circumstances which have created difficulties in identifying species of *Fusarium* are similar to those previously referred to in this *Bulletin* when discussing *Penicillium*, *Aspergillus*, and other hyphomycetus fungi. In Atanasoff's latest work he makes reference to this difficulty and points out "that most authors, whilst studying *Fusarium* species and not being able to tell whether their fungus had already been described or not, considered it a new one and described it as such, but always in a way that no one could identify again their organism with their description of same. Others following the old mycological practice did something even worse: when finding some *Fusarium* species on a certain plant, they simply looked up the literature, and if perchance there had been already described some *Fusarium* species as a parasite of this plant, then they simply used the name of this species for their organism. Others, again, being ignorant of the nature of *Fusarium* species, used the name "*roseum*" for any species which caused pink or rose-coloured discolourations and thus placed under one name a large number of widely different species."

An important result of this intensive study of the *Fusaria* has been to eliminate a considerable number of ill-defined species bearing many names and to classify within the genus ninety-two species which have been adequately described. A minor result, but one of special bearing on the subject of the present article, is the disappearance of all the names above-mentioned in connection with the red mould of barley, with the exception of the generic name, *Fusarium*.

Appel and Wollenweber discard the following five genera on grounds of synonymy with *Fusarium*: *Atractium*, *Fusidium*, *Fusisporium*, *Fusoma* and *Pionnotes*; the latter name in the form, *Eupionnotes*, being retained for a *Section* of *Fusarium* in which the species are specially characterised by the production of *pionnotes*. On that account the *Fusisporium* of Worthington G. Smith, and Lindner, as well as the name *Selenosporium* used by the latter author, are invalid.

Among specific names, *graminearum*, as used by Matthews, is now known to belong to the *Fusarium* stage of *Gibberella Saubinetii* (Mont.) Sacc., quite a different fungus. Smith's *F. hordei* is a name stated by Atanasoff to have been applied to some fungus different from that described by Matthews. *F. heterosporum* Nees., to which Massee transferred the red corn mould, is a synonym of *F. herbarum* (Corda) Fr. The specific name *moschatum* used by Lindner is not recognised by Wollenweber in his "Conspectus Analyticus Fusariorum," of 1917, and as pointed out by Lott, it cannot represent the fungus found in the malt-houses of this country. *Selenosporium aquæductum* Lagh., which Lindner thinks may be identifiable with *F. moschatum*, is *Fusarium aquæductum* Lagh., according to Wollenweber, and is a species characterised by 1-septate conidia, which are 30—15 μ shorter than the conidia of the common red mould of barley-corns. The conidia of the latter are 3—6, mostly 5-septate.

Although 40 years have elapsed since the red mould of barley was first described it is tolerably certain that only one mycologist has succeeded in identifying and giving an adequate reason for the identification of the species that Matthews had under observation; and he only within the last two or three years. Reference is

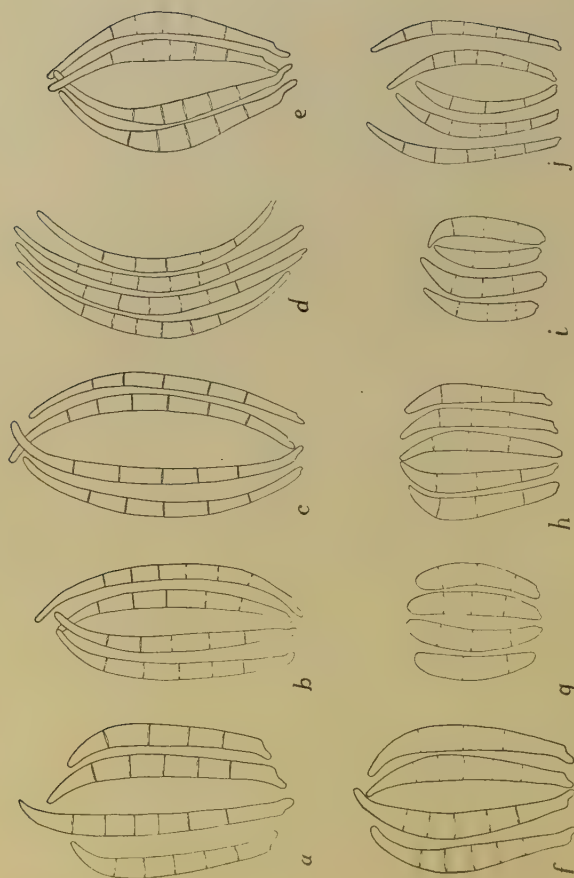
here made made to Dr. D. Atanasoff. With the publication of his recent monograph the uncertainty which has always surrounded the identity of this organism disappears. There is internal evidence in his work that recognition of the species only became possible as a result of patient detailed studies of the fungi belonging to the genus *Fusarium*; studies which have extended over a period of six years, and have included investigations carried out on two continents. The monograph contains much information of value to the barley buyer and maltster, although, primarily, it is of interest to the plant pathologist.

The identification of *F. hordei* appears to be quite incidental among the great mass of facts brought forward, which makes it all the more desirable that emphasis be given here to this point of special interest to fermentologists. In a brief reference to Matthew's paper of 1883, Atanasoff says: "Judging from his fine and most carefully executed illustrations of the fungus he had to deal with, there is little doubt but that it was *Fusarium herbarum*."

In a paper on "Fungi and the Fungus Diseases of Barley and Malt," (7) published in 1922, the present writer, in dealing with the question of the red mould of the malting floor, made reference to the earlier American work of Atanasoff, and therein suggested that the fungus then still known to brewers as *Fusarium hordei* was probably the imperfect stage of *Gibberella Saubinetii*. That suggestion was based on observations that *Fusarium* conidia of the type minutely described by Atanasoff had been found on barley-corns examined in the laboratories of this Bureau, as frequently as those which could be referred to any other species. Moreover, the fungus in the same stage had been collected in Yorkshire from stubble in the field, although, the presence of the perithecial, or perfect stage of the fungus, *G. Saubinetii*, had never been observed by the writer. As will have been gathered, this suggested relationship is not borne out by recent investigations, and the reason for a misinterpretation of observed facts is now available. From the work of Atanasoff it is abundantly clear that the following fungi,

Fusarium herbarum (Corda) Fr.

F. culmorum (W. G. Sm.) Sacc.



CONIDIA OF *FUSARIUM* SPECIES OCCURRING ON CEREALS.
(AFTER D. ATANASOFF).

- (a) *Gibberella Saulinetti* (Mon.) Sacc.
- (b) *Fusarium herbarum* (Corda) Fr.
- (c) *Fusarium avenaceum* (Fr.) Sacc.
- (d) *F. arcuoporum* Sherb.
- (e) *F. scirpi* Lamb. et Fautr.
- (f) *F. culmorum* (W. G. Sm.) Sacc.
- (g) *F. solani* (Mart. pr. p.) Ap. et Wr.
- (h) *Fredolens* Wr.
- (i) *Calonectria graminicola* (Berk. et Br.) Wr.
- (j) *F. arthrosporioides* Sherb.

F. avenaceum (Fr.) Sacc.

F. scirpi Lamb. et Fautr.

F. redolens Wr.

Calonectria graminicola (Berk. et Br) Wr.,

as well as the *Fusarium* stage of *Gibberella Saubinetii* (Mont.) Sacc., may all occur on barley-corns, inasmuch as they are all fungi parasitic on barley in the field.

All the species likely to be met with in English maltings are included in this list. The first two, and the last species named, have been found in the course of our own routine-work in malting and brewing practice.

None of these fungi are specific to the barley plant, and they occur on a number of hosts other than the cereals. As an aid in their identification, figures of the conidia are reproduced from Atanasoff by the courteous permission of Prof. H. M. Quanjer, Director of the Laboratorium vor Mycologie en Aardappelonderzoek, Wageningen.

The illustrations include the conidia of three species not previously mentioned, viz.: *F. solani*, *F. redolens*, and *F. arthrosporioides*. Although these have not so far been found on barley, they may at any time make their appearance on that plant, in view of the readiness with which these disease organisms attack any one of the cereals.

The presence of red mould on the malting-floor in any quantity is an indication that the crop from which it has been derived has suffered from one or other of the various forms of disease produced by *Fusarium* species. These may be root, foot, or stem-rot, or head-blight respectively. Atanasoff says, with special reference to the symptoms of blight-attack on barley heads: "They are usually different from those on wheat and rye. Only very seldom do they resemble those of the latter. Usually, only one kernel is killed, or several kernels in one row. In some cases, the three kernels forming a spiklet are attacked, and then the rest of the head, if conditions are favourable. The first sign of infection is a small water-soaked, somewhat brownish, spot at the base or the middle of the kernel, or it may appear first on

the rachis. The water-soaking and browning spreads in all directions from the point of infection, soon inclosing the whole kernel, the whole spikelet, or several spikelets, but this is in no way as uniform as it is in wheat and rye."

Conidia from either head-blight or stalk-rot are dispersed among the grain during threshing operations, and their subsequent development on the malting-floor, as described in the earlier paragraph, proceeds exactly as it would on barley sown in the field.

Many of the conidia from a diseased crop overwinter in the soil and thus furnish a source of infection for subsequent crops: these conidia may give rise to seedling-blight, or the surviving plants may later be attacked by root-rot; if not killed at that stage they may afterwards succumb to stalk-rot, which spreads to the ear and gives head-blight. The point of importance to the barley buyer and maltster, is that the disease not only leads to diminished yield, but to serious impoverishment of the grain which is harvested. Our own experience with barley, showing any marked contamination with red mould, is that it gives low germinative capacity and uneven growth on the floor.

F. A. M.

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Brewery Flies

IN searching the files of our monthly contemporaries devoted to brewing interests we have been struck with the frequency of a note of warning under the stereotyped heading, "Mould in the Malthouse." We have looked in vain for any note which might as regularly be sounded on the theme, "Flies in the Brewery," and we are not a little puzzled to understand the reason for the omission. The possibility of the transmission of infective fermentation organisms by insects appears to have received astonishingly little attention. The "Collective Index" to the *Journal of the Institute of Brewing*, which covers the literature of the industry over a period of 23 years, contains no reference either to "Fly" or to "Flies." Yet, the micro-biologist and the pathologist are keenly alive to the extraordinary capacity possessed by the common house-fly,¹ the latrine fly,² and the stable fly³ for inoculating food materials with organisms native to the filth in which each breeds. An experiment usually carried out in an elementary course of bacteriology is that in which a fly is allowed to walk across the surface of a nutrient medium, generally consisting of beef broth agar, placed in a petri dish, after which, the medium is incubated. After about 48 hours at a temperature of 37°C., closely-packed colonies of various species of bacteria mark the path followed by the fly in its perigrinations over the agar. If a similar experiment is performed using beerwort agar as the nutrient substratum the subsequent colonies consist chiefly of various species of yeasts, torulæ, and moulds, the two experiments indicating that a considerable micro-biologic flora of diverse character may be derived from the visits of a fly.

Literature on the subject of the ordinary domestic flies and their dangers is often freely distributed and is otherwise readily available, and there is no need to refer further to the several species mentioned above. Particulars regarding the little brown, dipterous insects which specially haunt the brewery, cider mill, and other

¹ *Musca domestica*. ² *Fannia scalaris*. ³ *Stomoxys calcitrans*.

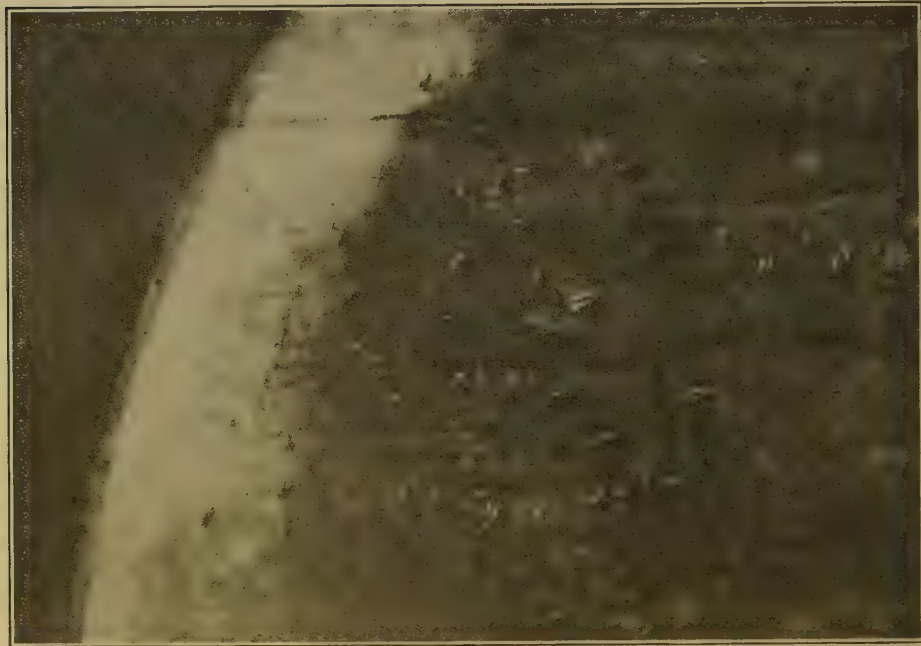


FIG. 23.
CASK HEAD, SHOWING PUPARIA OF VINEGAR FLIES.



FIG. 24.
PUPARIUM OF VINEGAR FLY $\times 12$.



FIG. 25.
DROSOPHILA AMPELOPHILA 8
(AFTER HOWARD).

works are not so readily found, and it is these insects which form the subject of the following notes.

Brewers are familiar with small, brown, red- or golden-eyed flies, varying in size from about one-third to half that of the common house-fly. They are to be observed about the gravity cylinders or fining glasses; they frequent the laboratory and may be found floating in malt mashes left standing overnight in a beaker, as well as being frequently seen flitting about the tun room and cellar. A close examination of the grains pit in Summer will usually result in the location of specimens. These insects belong to a group popularly known as "fruit flies," "vinegar flies," "pomace flies," etc. They subsist chiefly on saccharine matter as represented by ripe and decaying fruit, beer-wort, vinegar-wort and vinegar, wine-must, brewers' grains and many other vegetable substances; they may also be found breeding in animal excreta and other refuse. The scientific name of the family to which they belong is *Drosophila*, a name which has now become familiar to readers of the scientific press in connection with a phase of experimental biology. Flies belonging to this family are reared in countless thousands—in America—by students of genetics who are interested in the elusive chromosomes of certain cells rather than in the obvious infective character of the whole insect.

There are at least three species commonly found in this country: *Drosophila funebris*, *D. fenestrarum* and *D. melanogaster*. Specimens of the latter insect were sent to us from a London brewery during the Summer of last year, when they were very abundant. A common species in America, met with particularly in the fruit-canning industry, is *D. ampelophila*, and as the several species only differ in minute details we make use of a drawing of the latter from an authentic source in illustration of the perfect insect and its larva, Fig. 25.

The life-history of the vinegar fly proceeds in stages similar to those of the house-fly. The insect lays something like fifty eggs, for the deposition of which is selected some out-of-the-way place in a position plentifully supplied with food for the future larvæ. The eggs quickly hatch out into minute legless grubs which feed continuously for a fortnight, when they pupate, in which state

they remain for a week to a fortnight, according to atmospheric conditions, before emerging as flies.

The interiors of dirty casks are frequently selected as breeding haunts. Our illustration, Fig. 23, from a photograph taken in this Bureau, depicts the condition of the inside surface of a head taken from a cask which had been thrown aside as a "stinker." As may be seen, the underside of the head (only a portion of which is shown) was more or less covered with the strongly adhering puparia of these flies. A photomicrograph of a puparium after removal from the cask head is shown in Fig. 24. The flies, on emergence from puparia occupying such a position, would find themselves among yeasts, torulæ, mycodermæ and acetifying bacteria with which they would be bound to become infested, and it requires little imagination to appreciate the possibilities of infection latent in a horde of flies liberated from such environment.

The measures generally accepted as necessary for the control of the fly population and its attendant dangers have two objects:

1. To prevent the flies from breeding.
2. To prevent the transference of infection by them.

The removal of all material suitable for the development of the fly larvæ is of fundamental importance from the point of view of breeding prevention. Special watch should be kept for any drip of wort or splash of priming, etc., into corners readily overlooked but easily accessible to the flies and which would serve their purpose admirably. Manure heaps should not be allowed to stand in the vicinity of a brewery for any length of time exceeding one week in Summer, or more than nine days at any other time of the year. The grain pit should be kept clean and covered.

One can only be quite certain that a fly will not be the means of transmitting infection when the fly is dead. Vinegar flies, as well as flies of most species, can be baited and killed in the brewery by placing in various situations shallow dishes containing wort to which has been added five per cent. of Formoxil—about a teaspoonful to a soup-plate of wort. Flies are attracted to this solution on entry into the brewery and are thus prevented from doing mischief.

F. A. M.

The Suppression of Insect Pests and Fungoid Diseases

5. BED-BUGS (*CIMEX LECTULARIUS*)

THESE insects have been more or less tolerated as being harmless, if unpleasant, creatures. This may be the case as regards this country, where they are usually associated with dirty slum dwellings, and where cleanliness is not intimately associated with godliness.

In tropical and sub-tropical lands, however, they are sources of real danger; firstly, owing to their natural habitats, and secondly, as carriers and disseminators of disease organisms. Luckily here we are not likely to be troubled with tropical diseases, thanks to the untiring energies of the Port Medical Officers of Health, and our ever-increasing knowledge of up-to-date methods of sanitation, together with the spreading of the gospel of the principles of personal and state hygiene.

Nevertheless, if the bed-bug were allowed a free run, it might prove a very serious menace to the health of people, particularly those living in the industrial and thickly populated areas.

These insects in the adult stage can move fairly rapidly, but appear to easily lose their hold, which accounts for them falling from the ceilings on to any unfortunate person below.

They feed on man and other mammals, including and under artificial conditions, domestic animals, mice and rats, by sucking the blood after having punctured the skin. They appear to bite

indiscriminately, consequently some people appear to suffer more inconvenience than others.

According to Klingmüller, odour does not appear to guide them in their search for blood, and he states that their saliva may contain a substance which prevents the coagulation of blood, and this, with the mechanical injury produced by biting, may account for the serious inconvenience sometimes produced.

LIFE-HISTORY.

According to Blacklock, the average incubation period of the eggs at laboratory temperature was 17 days during June, July and August—14 days at 22° C. and 10 days at 25° C. Twenty-four hours immersion in cold water prevented them from hatching.

S. B. Doten points out that eggs kept in cold storage for 3 months, as well as newly-hatched larvæ (without food), promptly emerged when the temperature was raised. According to A. Hase, the eggs of *C. lectularius* are laid for some time after the female has begun fasting, and he states that at 94-99° F. none are laid after 7 days, which period increases to 12 days at 71-78° F., 27 days at 59-65° F. Up to 23 eggs are laid by a single fasting female, and, under optimum conditions, a large number of eggs may be laid by young females that have been once fertilised. Observations by the same author show a maximum number of 153 eggs in 36 days, the largest number of eggs laid in 24 hours being 12, and the maximum number for a single individual in her entire lifetime being 250.

The larval period may be shortened to 27 days by abundant food and an optimum temperature of 85° F.

Moulting may be delayed for months if these factors are lacking.

Klingmüller states that before reaching maturity the bug undergoes 5 moults at intervals ranging from 10 days to several months.

Apparently the larvæ begin to feed within an hour or two of emergence, but they cannot exist long at high temperatures.

Blacklock states that in his experiments, 150 unfed bugs completely succumbed at a temperature of 45° C. for 5 minutes. W. A. Bacot, in his experiments on the influence of temperature, submersion and burial on the survival of ova and larvæ of *C. lectularius*, states that the former survived exposure for 31 days at 40-50° F., and at temperatures of 28-32° F. for 48 hours; when exposed to the lower ranges of temperature for 5-8 days only 25% of the eggs hatched out, and 10-15 days proved fatal. Temperatures of 60-98° F. are favourable, but 113° F. prevents hatching.

Submergence in water between 60-63° F. for five days has no effect upon hatching if maintained afterwards under favourable conditions.

He (Bacot) states that newly-hatched bugs, when unfed, can survive a temperature of 28-32° F. for periods up to 18 days, and can withstand chilling, thawing and re-chilling, and again thawing for shorter periods.

When subject to cold moist air after a full meal, they are liable to a heavy, or even total mortality, probably in consequence of humidity rather than cold.

Between 60-65° F. they may live for 136 days unfed, and after a meal for 9 months. At 75° F. unfed bugs lived on an average of 10 days when the humidity was between '65 and '7. He found that 113° F. was fatal within a few minutes.

Investigations carried out by C. L. Marlatt indicate that the bugs may gain access to any house in spite of the adoption of all reasonable precautions. When its source of food supply ceases in any one place, it develops migratory habits, and escaping by windows, may pass along walls, water-pipes, gutters, etc., and thus gain access to adjoining houses. The same author states that it becomes quiescent and partly hibernates at temperatures below 60°, whilst at temperatures between 60-98° F. it is most active.

In heated houses the pest may remain active throughout the winter, and in the U.S.A. it is preyed upon by the house centipede (*Scutigera forceps*), and the red house Ant (*Monomorium pharaoni*).

CONTROL METHODS.

Various methods have been either suggested or tried by different authors. These include physical, such as excessive heat and moisture; chemical, which embrace contact sprays, asphyxiants and fumigants. The particular method to be adopted in any case will largely depend upon whether the houses or premises are inhabited or otherwise. In the former case it would for obvious reasons be impossible to attempt to seal off a portion of a house after fumigation with hydrocyanic acid gas, without very serious risk of endangering the lives of the occupants.

Whilst cyanide gas is extremely effective, providing the concentration is sufficient, and the fumigation is maintained for sufficient time, yet it is a very dangerous gas to be handled by inexperienced persons. There have been quite a number of fatalities recently where ships' holds have been fumigated with this gas against insect pests, rats and mice, and for this reason alone, and especially if other equally effective but less dangerous products are available, its use should be deprecated and discontinued.

In empty houses undoubtedly fumigation is the only effective method for complete eradication.

Recently the writer has had occasion to carry out some experiments on the elimination of bed-bugs on a housing estate in London.

Various fumigants had previously been tried by the authorities concerned, including sulphur, but without effect. There seems little doubt concerning the tenacity of life of these insects, and only drastic methods with potent insecticides are of any use in effecting a complete control.

During these experiments numerous volatile substances were used, including our "Fumesco," which is an effective specific against the Tomato White Fly (*Aleurodes vaporariorum*). This product was used at the rate of 20 fl. ozs. per 1,000 cu. ft. of space, but after 48 hours fumigation about 25% were actually killed. This fumigant, however, possessed a peculiar yet distinct advantage,

inasmuch as it caused the bugs to leave their places of hibernation, presumably in search of air. They certainly did not like the vapours of the fumigant.

In another experiment the temperature of the house was raised by lighting fires in each room prior to fumigation, the object being to make the bugs more active. All windows, doors, fireplaces, gratings and ventilators were then securely pasted up with stout paper, and sacks were suspended in each room. An application of our Alvesco Grain Fumigant, which has given such excellent results in controlling grain pests in malt-houses, etc., was made, using 10 fl. ozs. per 1,000 cu. ft. of space, and the remaining exit effectively sealed, the liquid being poured on to the suspended sacks in order to facilitate rapid evaporation. The premises were sealed for 48 hours. On opening out, most of the fumes had dispersed, so that very little unpleasant effects were experienced on entering.

On a close inspection, only 5 live bugs were found, but in such positions as to indicate that the vapours had not reached in sufficient concentration to kill. Five days afterwards 30 live bugs were found, but no further catches were made after this date. The floors were literally strewn with their bodies, and a representative selection was exposed in the warm sunshine for 2 hours, but there was no indication of recovery in any of the specimens under observation.

In future trials it was decided to use 20 ozs. per 1,000 cu. ft. (that is double the previous concentration), owing to the excessive leakage that had taken place, presumably due to the porosity of the breeze blocks used in the construction of the house. The great advantages with this fumigant lie in the fact that:—

- 1.—It is quite effective in killing larvæ and adults in one application, and there is reason to believe that it may possibly kill the eggs at the same time, since no larvæ have subsequently been seen, and the house previously was practically alive with the pest.

- 2.—The vapours in concentration irritate the eyes and mucous membranes of the nose and throat, and this is a valuable danger signal not possessed by cyanide gas.

3.—The fumigant is easily applied and the evaporation is not so rapid as to make its application dangerous to the operator. (No masks were worn during these experiments). It is also non-inflammable.

4.—The liquid can be put up in 1 pint bottles, so facilitating easy application.

5.—No acids, dishes or apparatus of any kind are necessary, thus saving time and labour.

The fumigant, although perhaps more expensive than cyaniding, can be used in semi-skilled hands, and there is not the same risk to the operator, providing all the preliminary sealing-up of the premises is carried out beforehand. Many contact sprays have been used for spraying against bed-bugs, but these can be of little use unless they actually hit the bug, a process not always easy to accomplish. Experiments are, however, in progress on these lines with a view of producing something which can be applied in inhabited houses, by spraying cracks and crevices, spaces between floor boards, wainscoting and picture rails, and so kill the pest in its place of hibernation. If such a product is produced, it will necessitate repeated applications to be really effective.

In 1912, B. Blacklock published a paper in *Ann. Trop. Med. Parasit.* VI., No. 4, in which is described numerous powder preparations which were experimented with, but their action in killing appears to be to a very great extent mechanical. He states that Dalmatian Flower Powder and fine French Chalk are good insecticides and that Cinchonæ quickly renders the bugs incapable of progression.

A 5% solution of Carbolic Acid killed in 10 minutes, and a 10% solution killed in 2 minutes. A one minute exposure killed about 80%.

A 1-1000 solution of Mercuric Chloride proved ineffective after two hours immersion.

Immersion in Paraffin Oil proved very effective after 5 secs., all the bugs dying within 24 hours, and the author regards this material as the most efficient of all the liquids tried.

T. P.

Comparison of Three Commercial Brands of Arsenate of Lead

Up to two years ago very little dry Arsenate of Lead was used in this country, and the small quantities that were in use being employed in conjunction with sulphur for dusting on a small scale.

Fruit growers do not seem to have been impressed with the use of arsenate dusts for use against leaf-eating insects, and have consequently kept to the paste forms which usually contain 50% moisture. This apparent prejudice against the use of arsenate dusts is to a considerable extent justified for the following reasons:

In comparison with conditions in the United States and Canada the areas here which have to be treated are much smaller, consequently greater care would have to be exercised to keep the dust within the confined limits. There would also be considerable risk of contaminating ground-crops with arsenate where these are grown between the tree rows in plantations or grass in the case of orchards. Then there are the important questions of endangering the health of people living in close proximity to and surrounding the fruit areas, as well as that of the operators.

Many brands of powder arsenate do not readily take up in water unless first mixed into a cream prior to diluting down. This disadvantage has, however, been overcome in the process of manufacture, but as will be shewn later there is by no means uniformity in physical characters among the various commercial brands.

In the case of arsenates for dusting, the important properties are:

1. The fineness in state of sub-division, which will enable the dust to adhere.
2. Floating capacity, *i.e.*, the density of the particles, must be sufficiently light to enable a dust fog to remain as such when delivered from a suitable distributing machine.

The important points in a powder arsenate which is to be used in water suspensions are:

1. Fineness in sub-division and lightness in density of particles.
2. Spread well when applied to young foliage.
3. Readily mix with water and remain in suspension for at least half-an-hour without settling.

In a recent investigation carried out by the U.S.A. Bureau of Chemistry and Entomology, Department of Agriculture (*Bull.* 1147, Cook and McIndoo), some important conclusions have been obtained on the chemical, physical, and insecticidal properties of arsenicals that are at present on the market.

It has been found that the toxicity of As_2O_3 varies greatly, and is dependent upon the degree of fineness of the crystals, which influence the percentage of water-soluble As_2O_3 present. Acid lead arsenate may be recommended in general when an uncombined arsenical is to be used. It is said to possess excellent adhesive and insecticidal properties, and causes very little, if any, scorching of the foliage. It is compatible with nicotine sulphate, and Bordeaux mixture, but not with lime sulphur solutions, at least from the chemical standpoint.

A powdered lead arsenate contains about 32% As_2O_5 and 64% lead oxide, while powdered basic lead arsenate contains about 23% As_2O_5 and 73% lead oxide.

The alkalis in soap tend to decompose lead arsenate, although with calcium arsenate a smaller amount of soluble arsenic is formed if used with a soda-fish-oil soap, than in the

case of acid lead arsenate; this latter arsenate should not be used with paraffin emulsions, as the mixture is obviously chemically incompatible.

It has also been shown that soluble arsenates are more toxic per unit of arsenic than those which are insoluble; the greater toxicity being due to the water-soluble arsenic in the compound, or the arsenic that is quickly made soluble in the digestive tracts of the insect.

Apparently it is not always true that an arsenate with a high percentage of arsenic is more toxic than one containing a lower percentage, it seems to point to the fact that toxicity depends not alone upon the arsenic present, but also upon its form of combination. The addition of lime or Bordeaux mixture to arsenicals reduces their toxicity, the calcium present apparently preventing or counteracting the formation of soluble or more toxic arsenic compounds.

The rapidity with which the arsenicals are made soluble in the body of the insect seems to be the most important factor in connection with their toxicity. Part of the soluble arsenic inside the body of the insect passes through the intestinal walls into the blood and is distributed to all parts of the body, a small quantity reaches the nervous system, but what happens to the remainder is not known.

The insecticidal part played by the base itself appears to be small and sometimes non-existent. It appears that the chemical evaluation of arsenates does not give sufficient data to judge of their insecticidal value, and a toxicity study alone does not show that an arsenical is suitable for general insecticidal purposes. It is necessary, in order to obtain satisfactory evidence, to make a study of both properties.

"Arsenical Injury."—Swingle, Morris and Burke (*J. Agric. Res.*, XXIV, No. 6) state that the arsenical insecticides least injurious to foliage are ferrous arsenate and certain arsenates of lead. All pure diplumbic ortho-lead arsenates are not identical in burning properties, nor are triplumbic ortho-lead arsenates identical in this respect.

Some "diplumbics" are as safe to use as some of the "triplumbics." Arsenic as trioxide is stated not to be as dangerous to foliage as is generally supposed, and this insecticide is permissible on any but the most delicate foliage, provided it is applied promptly after mixing with water.

Standing after mixing causes a very marked increase in injury by arsenic trioxide, and a slight increase in injury by other arsenical insecticides.

Of the readily soluble arsenical compounds, cacodylic acid, and the sodium and potassium salts of this acid, have been proved to be the most injurious. The foliage appears to be more susceptible to arsenical injury than the fruit or the stems. The injury to the foliage is practically all through the lower epidermis, regardless of the number of stomata in the two surfaces, indicating that it is a result of direct penetration of the thinner cuticle. Individual plants of the same species and variety appear to vary somewhat in their susceptibility to arsenical injury. The older leaves being more susceptible than the younger ones.

Soap added to most insoluble arsenicals increases the injury by increasing the solubility to a point more than counteracting its slight protective action, while soap added to soluble arsenates offers a slight protective action. Soap added to Paris green in suspension distinctly restrains the burning of the foliage. Gelatine, milk and agar do not increase the arsenical injury to foliage. Lime sulphur seems to increase the injury caused by most soluble arsenates while this material distinctly decreases the injury when used with calcium arsenate, but not sufficiently to make this material a safe insecticide.

Light does not appear to be an important factor in arsenical injury to foliage, while an increase in atmospheric temperature results in an increase in injury. If the air is relatively dry, this temperature effect is not of great practical importance. Humidity is the greatest environmental factor in determining arsenical injury to foliage, and this influence is very marked, even before the saturation point is reached.

"Action of Soap upon Lead Arsenate."—Pinckney (*J. Agri. Res.*, XXIV, No. 1) considers that the soap dissolves the arsenic from the acid and neutral lead arsenate, and may cause injury to the foliage.

This experience has been confirmed in our laboratories, the action being much more pronounced if the soap is not neutral (*i.e.*, containing free alkali). The stearate soaps appear to have a greater dissolving action than the oleate soaps, particularly in the case of the "acid" arsenate.

For this reason we have made it a practice to deprecate the use of soap with arsenate of lead, in spite of the fact that many growers here are in the habit of using a nicotine soap, and arsenate of lead spray in the early spring. In this respect an alkaline hard water is liable to cause arsenical injury if used with acid lead arsenate.

Experiment 1.—Density.

Equal weights of three commercial brands of lead arsenate, No. 1, 2, and 3, were weighed out and placed in glass tubes of equal size and dimensions. The tubes were gently tapped to secure maximum settling down. These were photographed and are shown on the left-hand side of the watch in Fig. A. They were placed in numerical order as above indicated.

It will be observed that there is a considerable difference in bulkiness or density.

Experiment 2.—Suspensions in Water.

Suspensions of each brand, at the rate of 2 lbs. per 100 gallons of water, were made and placed in cylinders of equal size and dimensions in numerical order, as in Experiment 1. All were shaken up at the same time for 30 seconds, and allowed to stand for five minutes. A photograph was then taken; and again after a period of standing of 20 minutes, one hour, and five hours. It will be noticed that Nos. 1 and 2 showed settling out after 20 minutes' standing, and almost complete settling after one hour, while at the end of five



FIG. 26.
SUSPENSION TEST ON THREE SAMPLES OF LEAD ARSENATE.



FIG. 27.
PHOTO-MICROGRAPHS SHOWING RELATIVE SIZE OF PARTICLES.

hours' standing Brand No. 3 was only beginning to show signs of settling, Fig. 26.

Experiment 3.—Fineness in State of Division.

Microscope slides were prepared with "drop suspensions" of the three brands (at the rate of 2 lbs. to 100 gallons of water), and photo-micrographs were secured as shown in Fig. 27, the plates being again in numerical order. It will be seen that there is a great difference in the size and uniformity in size of the particles as well as their distribution over the plate surface, No. 3 being the best from this point of view.

Experiment 4.—Spreading.

Spreading tests were carried out with suspensions of 6 lbs. per 100 gallons of water, in each case using a "Benton and Stone" mist nozzle. The spray was delivered at 40 lbs. pressure per inch at the nozzle aperture, and the distance of the nozzle from the test plate being 18 inches. The results are illustrated in Fig. 28.

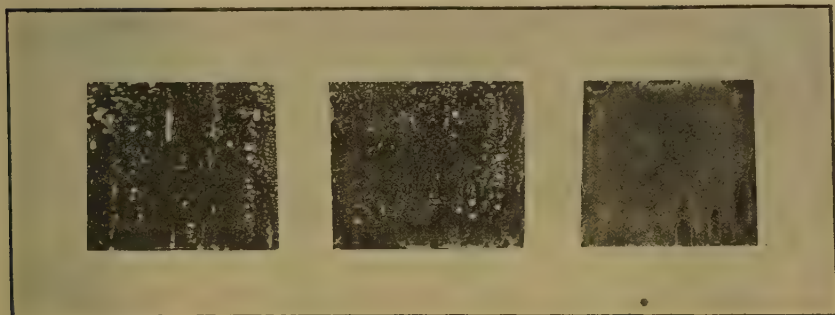


FIG. 28.—SPREADING TESTS ON GLASS PLATES.

Similar experiments were performed, using a "Mistifier Junior," nozzle No. 1, aperture plate at 18" distance.

The test plates that were used consisted of highly-polished glass sheets (polished with flannel and silk).

NOTE.—It was found that greased plates, dipped in a 0·2% solution of mineral jelly in petrol, offered a surface of too high a resistance to make good comparisons. The results are self-explanatory, No. 3 Brand giving good spreading.

ANALYSES OF TEST SAMPLES.

	AS ₂ O ₅	AS ₂ O ₃	Lead Oxide (PbO)
Brand No 1	30·0·%	·5%	60—62%
Brand No 2	30·0·%	Less than 1%	62%
Brand No. 3*... ..	30·0·%	·65%	60—62%

* This sample was stated to contain a deflocculator.

SUMMARY.

1. Although the arsenic pentoxide content of each brand was 30%, there appears to be a very great difference in physical properties.
2. There appears to be some correlation between (1) density and fineness of division; (2) suspensibility and fineness in division, and one would expect to find a similar correlation between fineness of division and spreading. But, of course, in the case of No. 3, the presence of a deflocculator must be taken into consideration.
3. The value of No. 3 brand needs little comment on its value from the growers' point of view. From the physical characters one would expect it to give much better killing results than either No. 1 or No. 2, on account of its fineness in state of division and spreading and suspension properties.
4. In determining the insecticidal value of arsenates, it appears to be important to study the chemical and physical properties, together with its actual killing properties on live insects.

The chemical properties give little indication of the insecticidal value, apart from the actual arsenic content.

T. P. AND A. W. L.

The Use of Emulsified Oils as Ovicides

IT has generally been assumed, at any rate in this country, that any chemical which could be used on growing trees, even in the dormant period, as a means for the destruction of insect eggs, at such a strength to be effective, would have a very detrimental effect upon the tree itself.

Experiments carried out on a field scale during the past season have, however, shown that this assumption is somewhat erroneous.

Since completing the above article, a report on "Egg Destroying Washes," by A. H. Lees, *Journ. of Pomology and Hort. Science*," Vol. III., No. 4, 1924, has been published. The author states that:

1. Lime Sulphur by itself is ineffective in destroying egg of *A. pomi*.
2. Lime Sulphur with 0.2% calc. caseinate has a strong killing action at 1—15, 1—20, and 1—30 dilutions applied in February or March.
3. Combinations of Caustic Soda and Nicotine have strong killing action if applied just prior to hatching.

Lime Sulphur, Caustic Soda, and Caustic Lime, have been extensively used during recent years in the dormant period, but it is very much open to question whether any or all have really substantiated the claims originally put forward as regards actual egg destruction. Unquestionably they are excellent specifics for

the general cleaning of the trees by the destruction of Moss, Lichen, etc., which act as hibernating quarters for both insect pests and winter spores of certain fungoid diseases.

Lime, when applied hot and freshly slaked, and as thick as it is possible to spray with, does appear to either retard or prevent the hatching-out of certain eggs. But cases have been frequently observed where the young larvæ have managed to extricate themselves from eggs apparently well covered with a good layer of Lime Wash. "Case hardening," however, cannot be taken as direct chemical destruction of the eggs. Again, Lime Sulphur Solution, although certainly caustic in its action, only appears to leave a finely-divided film deposit of sulphur which is not likely to disintegrate the chitinous covering of which the outer shell of the egg is composed. Also, with caustic soda, the final deposit after exposure to air will be carbonate of soda, which is readily soluble in water, so that causticity will rapidly disappear.

The strength at which the Caustic Soda is generally used would not be sufficient to cause a rapid breaking down of the egg chitin before carbonation takes place. It is quite possible with both Lime Sulphur and Caustic Soda that a stifling and case-hardening effect is produced which may either retard the ultimate hatching-out of the egg or even prevent this process taking place altogether as a result of the deposit formed on the egg shell.

According to Bloxham, chitin can be obtained by extracting the exoskeleton of a beetle with 5% HCl, hot alcohol, 5% KOH and water, drying, and again extracting with ether and dilute HCl. The chitin is a white translucent substance soluble in conc. H_2SO_4 . From this consideration it would be expected that an application of a 2½% solution of Caustic Soda applied as a Winter Wash, would have very little effect upon insect eggs. This assumption is corroborated by actual experience under field conditions.

In the States, during the last few years, considerable attention has been devoted to the use of Oil Emulsions, particularly for dormant spraying. The use of these Emulsions has been adopted not only for destruction of scale and hibernating insects, but as

a means of egg destruction. The class of oils used varies somewhat in character, but generally speaking those obtained from the distillation of tar seem to be favoured, probably on account of their plentiful supplies, and comparatively low cost of production.

It has been shown by various workers in the field of economic entomology that oils having a boiling point above 250°C show a gradual diminution in insecticidal value with increase in boiling point, but there is reason to believe that this property may be only characteristic of certain oils.

Recent work carried out has certainly indicated that such oils used for this class of work must be of a definite specification, and that all fractions of tar distillates are not necessarily efficient as egg destroyers.

Another extremely important factor is the complete emulsification of the oils, so that the resulting product is water miscible, otherwise there is considerable risk of subsequent damage to the trees. In the United States the concentration of oil emulsion to water for spraying purposes varies with the different make, but in all cases a much higher concentration is necessary than growers have hitherto used in this country, ranging from 5% to 10%.

There seems little doubt that some eggs are more resistant to this treatment than others, particularly those of the Winter Moth (*Cheimatobia brumata*). This may be due to the difference in thickness of the chitinous covering of the egg.

The action of these emulsions seem to indicate a gradual disintegration of the chitinous covering with absorption of the oils, thus causing a coagulation of the embryonic protoplasm.

T. P.

A Further Note on the Carnivorous Habits of *Tachycines asynamorus**

DURING the last eleven months we have had under observation a colony of the above insects in captivity. Throughout this time numerous attempts have been made to induce the insects to feed upon a variety of foodstuffs, but so far without any apparently conclusive results.

The insects, 14 in number, have been housed in a breeding cage, fitted with perforated zinc sides and a glass door, containing a tray of soil kept slightly moist, together with a small shallow drinking trough, the latter being daily filled with fresh water.

The insects appear to prefer the dark and have been continually seen, with few exceptions, resting in an inverted position on the top of the cage. Very occasionally they have been noticed at night-time at the drinking trough, but on approach to the cage, there has been a general stampede to the roof again. When the specimens were first obtained the body parts (*i.e.* head to anal tip of abdomen) measured not more than $\frac{3}{8}$ -ths of an inch, but at the time of writing the remaining specimens, four in number (three males and one female) measure approximately three-quarters of an inch in length. The female measuring slightly more.

Copulation has never been observed nor has there been any appearance at any time of young.

Small quantities of bread, meat, sugar, flour, lettuce, cabbage, young tomato seedlings and lobelia have been given to them in

* *Bull. Bur. Biotec.*, No. 8, Vol. 1, pp. 262—267.

turn, also *Aphis rumicis* on bean, ordinary green aphid, and a number of dipterous flies.

So far as one could observe, the bread, meat, sugar, flour, aphid and flies were left untouched, and among the vegetable matter given the lobelia appeared to be eaten to a very small extent, if at all. Any feeding that did take place must have occurred at night-time. It was noticed that a gradual diminution in numbers of the colony was slowly taking place, and this could not be accounted for, as no dead insects, or remnants of skins, extremities, etc., were ever found on the floor of the cage after very careful examination. In June of 1923, in the early evening, a female was observed in a dying condition, being dismembered by two others (sex undetermined, as they left the body at once on being disturbed). The body was removed to a glass slip and replaced in the cage in readiness for photographing, but before this operation could be carried out the body had been again attacked and was being devoured by at least five members of the colony on the roof of the cage.

This probably accounts for the gradual disappearance above indicated, and now there are only four remaining specimens; three disappeared during July, but no further casualties appear to have taken place since August 13th, 1923.

During September two further casualties occurred, and a further one in October. The remaining insect was observed to be a female, and after a further two or three weeks she died apparently from starvation.

From the results of these observations it is concluded that *T. asynamorus* when kept in captivity is liable to develop cannibalistic habits when unable to secure suitable vegetation. Further enquiries have shewn that the insect is a vegetable feeder when given free access to greenhouses or a commercial nursery. It is still causing trouble in certain nurseries, and the method of hunting by night with lanterns has given good results in keeping the pest in check.

T. P. and A. W. L.

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